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Clinical Value and Mechanism of Long Non-Coding RNA UCA1 in Acute Respiratory Distress Syndrome Induced by Cardiopulmonary Bypass



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Aim Long non-coding RNA (lncRNA) can be used as a biological marker for the diagnosis and treatment of various diseases. The study aimed to detect changes in the expression of lncRNA for urothelial carcinoma associated 1 (UCA1) in patients with cardiopulmonary bypass (CPB)-induced acute respiratory distress syndrome (ARDS). Clinical values and cell function in ARDS were explored.

Method In total, 195 patients without CPB-induced ARDS were included in the control group, and 85 patients with ARDS were included in the ARDS group. Serum UCA1 levels were measured by quantitative real-time polymerase chain reaction. A549 was used for the cell experiments by establishing oxygen–glucose deprivation/reperfusion (OGD/R) cell models, and the cell viability and apoptosis were tested. The concentration of inflammatory factors was tested by an enzyme-linked immunosorbent assay. A luciferase reporting assay was applied for target gene analysis.

Results Quantitative real-time polymerase chain reaction revealed a gradual increase in serum UCA1 in both control and ARDS cases, and patients with ARDS had higher levels of UCA1 than those in the control group. Serum UCA1 was positively correlated with serum tumour necrosis factor- α and interleukin-6 concentration in patients with ARDS. UCA1 had the ability to distinguish patients with ARDS from those without it. UCA1 inhibition protected against lung injury and inhibited cell inflammation in vitro. MicroRNA (miR-182-5p) was downregulated in OGD/R-induced cell models and sponged by UCA1.

Conclusions Elevated expression of UCA1 may be associated with the occurrence of ARDS after CPB surgery. The regulatory role of UCA1 in ARDS might be related to inflammation and downregulated miR-182-5p in alveolar epithelial cells.

Keywords UCA1/miR-182-5p • CPB • ARDS • Inflammation • Alveolar epithelial cell

Introduction

In recent years, the wide application of cardiopulmonary bypass (CPB) has greatly improved the success rate of

cardiac surgery. Although the postoperative monitoring technology for CPB is improving continuously, organ damage caused by CPB is still common and cannot be completely avoided [1]. The incidence of lung injury after CPB is as high

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as 12%–50%, and most of these patients require ventilator treatment, or even develop acute respiratory distress syndrome (ARDS) [2]. Once CPB patients develop ARDS, the fatality rate is 15%–68% [3]. In addition, ARDS will lead to prolonged hospitalisation and increased economic burden. Therefore, it is necessary to find more optimised CPB-related diagnostic indicators for ARDS.

Long non-coding RNAs (lncRNAs) are a class of transcripts of over 200 nucleotides in length. lncRNAs have a variety of biological functions and play a role in regulating the expression of specific genes [4]. Differential expression of lncRNAs has been widely identified in many human diseases [5]. Notably, studies on lncRNA in ischaemia–reperfusion tissue injury have been widely reported, including H19, metastasis associated lung adenocarcinoma transcript 1 (MALAT1), and nuclear paraspeckle assembly transcript 1 (NEAT1) [6–8]. High levels of urothelial carcinoma associated 1 (UCA1) were detected in pulmonary artery smooth muscle cells under hypoxia, which is further involved in promoting the development of pulmonary arterial hypertension [9]. UCA1 is also highly expressed in septic rats, which further aggravates lung inflammation and apoptosis, leading to pneumonia [10]. In lipopolysaccharide (LPS)-treated WI-38 cells, upregulation of UCA1 is also reported to aggravate LPS-induced cell apoptosis and the inflammatory response, ultimately affecting the development of pneumonia [11]. However, the role of UCA1 in CPB-induced ARDS has not been examined. The competitive endogenous RNA (ceRNA) networks hypothesis has been proposed in a variety of diseases, indicating that lncRNA can serve as a microRNA (miRNA) sponge or decoy to modulate the expression of miRNA targets [12]. Previously, miR-182-5p has been identified to be a target gene of UCA1 in several diseases [13,14]. miR-182-5p is a member of the miR-183/96/182 gene cluster [15]. In recent years, miR-182-5p has been reported to be abnormally expressed in inflammatory tissues and acts as an important regulatory factor in the inflammatory response. For example, miR-182-5p can inhibit the transcription of proinflammatory genes driven by tumour necrosis factor (TNF)- α [16,17]. More importantly, miR-182-5p may affect the proliferation, inflammation, and apoptosis of immune response cells related to the therapeutic effect of bone marrow mesenchymal stem cells in acute lung injury [18]. In addition, miR-182-5p is detected to be highly expressed in lung tissues and the bronchoalveolar lavage fluid of LPS-induced acute lung injury mice; its inhibitory effect on the release of inflammatory factors has also been confirmed *in vitro* [19]. It is also widely reported to be involved in the long disease, such as acute lung injury and lung cancer [20,21]. Therefore, in this study, we preliminarily explored the relationship between miR-182-5p and UCA1 in ARDS.

In this study, the expression of UCA1 in patients with CPB-induced ARDS was detected, and its association with the clinical data was evaluated. In addition, in light of the ceRNA between lncRNA and miRNAs, the underlying mechanism of UCA1 in ARDS with the involvement of miR-182-5p was further elucidated.

Materials and Methods

Study Subjects

A total of 280 patients who underwent CPB surgery at the Affiliated Hospital of Chengde Medical University were included in the study and divided into two groups according to the incidence of postoperative ARDS. Altogether, 195 patients without ARDS were included in the control group, and 85 patients with ARDS were included in the ARDS group. Patients with ARDS were diagnosed using the Berlin Definition criteria, which include bilateral lung infiltrates detected on chest radiographs, a pulmonary capillary pressure of ≤ 18 mmHg, and a partial pressure of O₂/fraction of inspired O₂ ≤ 200 mmHg [22]. The retrospective nature of this study makes it difficult to differentiate between ARDS, transfusion-related acute lung injury (TRALI), and transfusion-associated cardiac overload (TACO). We took all possible measures to exclude patients with TRALI and TACO from the ARDS group. We used expert panel criteria for the adjudication of non-acute post-transfusion hypoxaemia with bilateral pulmonary infiltrates to differentiate between TRALI, TACO, and ARDS, or other aetiologies such as pneumonia, aspiration, congestive heart failure, or diffuse pulmonary haemorrhage by chart review. Initial screening was done by two authors followed by adjudication by the senior author to minimise the risk of inaccuracy. The inclusion criteria of patients in this study were as follows: (1) underwent CPB surgical treatment, and received general anaesthesia under endotracheal intubation; (2) all patients in the observation group were diagnosed with ARDS; (3) no complicated diseases such as chest trauma, endocrine disease, liver and kidney dysfunction, and so on; (4) patients with a history of lung infection and chronic lung disease were excluded; (5) had no history of intravenous antibiotics prior to surgery for any active infections; (6) patients and their families volunteered to participate and signed an informed consent form; and (7) the experiment was approved by the Affiliated Hospital of Chengde Medical University Ethics Committee.

Serum Sample Collection

Five millilitres of peripheral venous blood was collected at four time points after thoracotomy, including before CPB (T0, after thoracotomy but before CPB), 4 hours after CPB (T1), 8 hours after CPB (T2), and 16 hours after CPB (T3). Serum was collected after centrifugation at 1,800 g for 10 minutes.

Cell Culture and Transfection

The human lung adenocarcinoma cell line A549 was purchased from the cell bank of the Chinese Academy of Sciences, Shanghai, and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum in a culture environment of 5% CO₂ at 37°C. Logarithmic-phase cells were selected for subsequent experiments. To mimic ARDS,

cells were cultured under anaerobic conditions in 85% N₂ for 4 hours, following 24 hours of normal conditions [23].

Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used for the cell transfection, according to the manufacturer's instructions. Sequences of small interfering RNA of UCA1 (si-UCA1) and its negative control (si-NC), miR-182-5p mimic or inhibitor, and their negative control (mimic-NC or inhibitor-NC) were constructed and synthesised by Gene Pharma (Shanghai, China).

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was done to detect RNA levels. Firstly, total RNA was extracted from serum samples or cells using Trizol (Invitrogen, Carlsbad, CA, USA), and transcribed into cDNA using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China) for UCA1 or a miRcute Plus miRNA First-Strand cDNA Kit (Tiangen) for miR-182-5p. Secondly, qRT-PCR was performed using a SuperReal PreMix Plus (SYBR Green) kit (Tiangen) for UCA1 or a miRcute Plus miRNA qPCR Kit (SYBR Green) (Tiangen) for miR-182-5p, according to the kit instruction leaflet. RNA levels were calculated via the $2^{-\Delta\Delta C_t}$ method.

Cell Counting Kit-8

Cell counting kit-8 (CCK-8) was performed to evaluate cell viability. Cell suspensions of each group were inoculated in 96-well plates at a density of 1,000 cells per well. CCK-8 (10 μ L) was added to each well at 0, 24, 48, and 72 hours after culture, and culture was continued for 4 hours. The optical density (OD) of each group of cells at 450 nm was measured and calculated with a microplate meter.

Flow Cytometry Assay

A flow cytometry assay was performed to assess cell apoptosis. Cells during the logarithmic growth were inoculated in 96-well plates. After different treatments, cells were collected and washed twice. Then, 5 μ L Annexin V and propidium iodide (PI) were added into the cells with gentle blending. Cell apoptosis was detected by flow cytometry after 15 minutes of incubation at room temperature.

Enzyme-Linked Immunosorbent Assay

An enzyme-linked immunosorbent assay (ELISA) was done to detect the concentrations of TNF- α and IL-6 in both serum and cell supernatant. The ELISA kits for TNF- α and IL-6 were purchased from tBlue Gene Biotech (Shanghai, China).

Luciferase Reporting Assay

LncBase Predicted v.2 (Sun Yat-sen University, Guangzhou, China) predicted the binding sites between lncRNA UCA1 and miR-182-5p, which was verified with a luciferase reporting assay. The wild type (wt) or mutant type (mut) seed region in UCA1 was synthesised by Gene Pharma (Shanghai, China), and cloned into the luciferase reporter

vector psiCHECK-2 (Promega, Madison, WI, USA). The vector was then transfected into A549 cells together with miR-182-5p mimic or inhibitor using lipofectamine 2000. After 48 hours of culture, luciferase activity was tested under a microplate reader (Molecular Devices, San Jose, CA, USA). Renilla luciferase was applied for normalisation.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 (IBM, Armonk, NY, USA). Measurement data were expressed as mean and standard deviation (SD), and compared for the difference between groups using a one-way analysis of variance. A chi square test was performed to analyse differences in the categorical variables. Correlation analysis was done using Pearson's correlation analysis. Diagnostic ability was assessed by establishing a receiver operating characteristic (ROC) curve. A p-value <0.05 was considered to be statistically significant.

Results

Basic Clinical Information of the Study Population

A total of 280 patients who underwent CPB surgery were collected; of these, 85 developed ARDS after surgery. All patients were placed in either the control group or the ARDS group, based on the onset of ARDS; clinical information was compared between the two groups. As shown in Table 1, the two groups were age- and sex-matched, and there was no significant difference in body mass index, indicating that the two groups were comparable. In addition, other conditions, including C reactive protein levels, reasons for surgery, preoperative cardiac function grading, operative category, and aorta blocking time, were also compared, and no significant differences were detected (Table 1). However, patients in the ARDS group had a long bypass time than those without ARDS ($p < 0.001$).

Long Non-Coding RNA Urothelial Carcinoma Associated 1 Levels in the Serum Patients with Acute Respiratory Distress Syndrome Patients After Cardiopulmonary Bypass

As shown in Figure 1, qRT-PCR revealed a gradual increase in serum UCA1 in both control and ARDS cases. However, at any time point before and after surgery, the serum UCA1 levels in the ARDS group were statistically significantly higher than in the control group ($p < 0.001$).

Correlation of Serum Urothelial Carcinoma Associated 1 with Inflammatory Cytokines

Tumour necrosis factor- α and IL-6 concentrations were detected in the serum of study patients, to evaluate the inflammatory response. The ELISA demonstrated an

Table 1 Basic clinical information of the study population.

	Controls (n=195)	Patients with ARDS (n=85)	P
Sex			0.996
Male	94 (48.2)	41 (48.2)	
Female	101 (51.8)	44 (51.8)	
Age (yr)	57.08±11.01	59.08±9.55	0.146
Weight (kg)	64.66±6.00	65.59±7.54	0.274
Height (m)	1.70±0.07	1.67±0.79	0.905
BMI (kg/m ²)	23.21±1.86	23.55±2.14	0.181
CRP	14.59±4.13	15.41±4.07	0.130
Causes of surgery			0.920
Rheumatic heart disease with valvular involvement	115 (59.0)	48 (56.5)	
Coronary heart disease	74 (38.0)	34 (40.0)	
Dissecting aneurysms	6 (3.1)	3 (3.5)	
NYHA class			0.697
II	19 (9.7)	6 (7.1)	
III	173 (88.7)	77 (90.6)	
IV	3 (1.5)	2 (2.3)	
Operative category			0.996
CABG	12 (6.1)	6 (7.1)	
Heart valve replacement	141 (72.3)	70 (82.3)	
Great vessels surgery	34 (17.4)	8 (9.4)	
Others	8 (4.1)	1 (1.2)	
CPB intraoperative situation			
Bypass	99.08±28.97	112.99±29.31	<0.001
Aorta blocking	67.31±26.69	73.19±26.15	0.090

Data are provided as n (%) or mean ± standard deviation.

Abbreviations: ARDS, acute respiratory distress syndrome; BMI, body mass index; CRP, C-reactive protein; NYHA, New York Heart Association; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass.

increasing trend for TNF- α and IL-6 release in the control and ARDS groups, in a time-dependent manner (Figure 2A, B). Patients with ARDS had higher levels of TNF- α and IL-6 than those in the control group (Figure 2A, B). In addition, Pearson's correlation analysis was done for the correlation analysis between UCA1 and TNF- α and IL-6 in the serum of patients with ARDS 8 hours after CPB. Serum UCA1 was positively correlated with serum TNF- α ($r=0.723$, $p<0.001$; Figure 2C) and IL-6 concentration ($r=0.800$, $p<0.001$; Figure 2D).

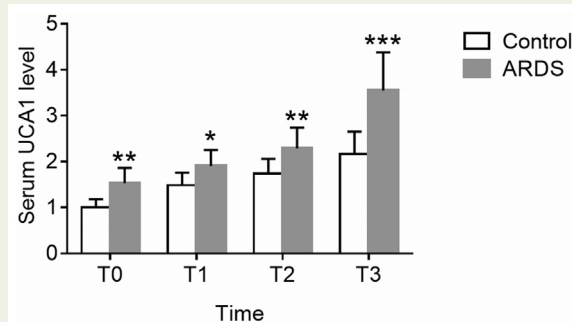


Figure 1 Long non-coding RNA urothelial carcinoma associated 1 (UCA1) levels in the serum of patients with acute respiratory distress syndrome (ARDS) after cardiopulmonary bypass. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ vs the control group.

Diagnostic Value of Serum Urothelial Carcinoma Associated 1

Using serum UCA1 level as the test variable and the occurrence of ARDS as the state variable, a ROC curve was drawn to evaluate the diagnostic value of UCA1 for ARDS. As shown in Figure 3, serum UCA1 can identify patients with ARDS from those who underwent CPB with the area under the curve of 0.934. The biggest Youden index was obtained at cut-off value of 1.211, with a sensitivity of 76.5% and a specificity of 96.4%.

Urothelial Carcinoma Associated 1 Inhibition Protects Against Lung Injury and Inhibits Cell Inflammation in vitro

OGD/R-induced cell models were established for in vitro experiments. Similarly to the results observed in patients with ARDS, high levels of UCA1 were also observed in the cell models, which was reversed by si-UCA1 transfection (Figure 4A). Cell counting kit-8 (CCK-8) demonstrated the cell viability inhibition induced by OGD/R treatments, and si-UCA1 rejuvenated cell viability (Figure 4B). In addition, OGD/R-induced cell apoptosis was recovered by UCA1 block down (Figure 4C). Cell inflammation was also evaluated. As shown in Figure 4(D), OGD/R promoted the release of both TNF- α and IL-6, and these effects were cancelled out by si-UCA1. The results demonstrated that si-UCA1 inhibited OGD/R-induced cell apoptosis and the release of TNF- α , but it did not recover to the levels of untreated cells.

Urothelial Carcinoma Associated 1 May Function Through Sponging miR-182-5p

The binding sites between UCA1 and miR-182-5p were predicted by LncBase Predicted v.2 (Figure 5A). In cells transfected with wt-UCA1, the luciferase assay demonstrated weakened cell luciferase activity, which co-transfected with the miR-182-5p mimic, while stronger luciferase activity was detected in cells co-transfected

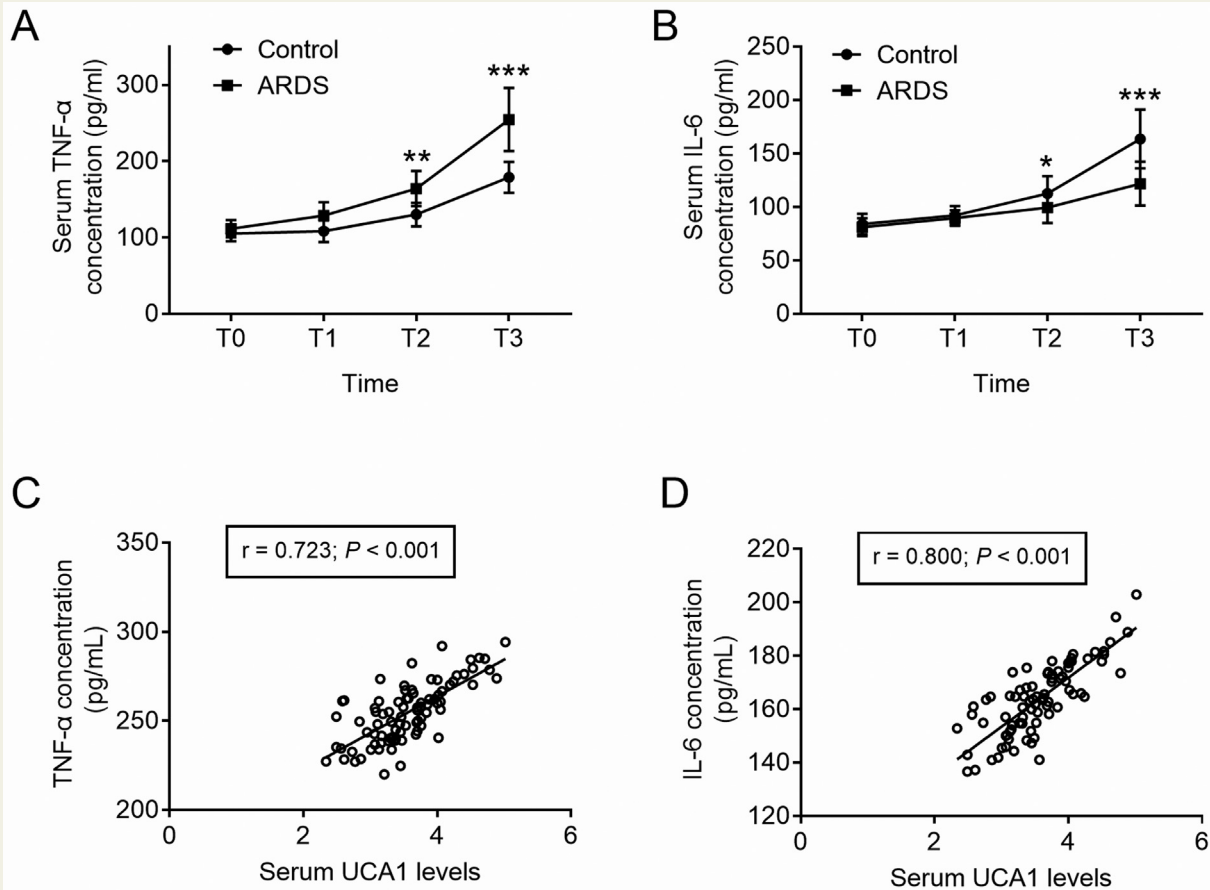


Figure 2 Correlation of serum urothelial carcinoma associated 1 (UCA1) with inflammatory cytokines. Patients with acute respiratory distress syndrome (ARDS) had increased levels of (A) tumour necrosis factor (TNF)- α and (B) interleukin (IL)-6 vs the control group. Serum UCA1 was positively correlated with serum (C) TNF- α and (D) IL-6 concentration. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

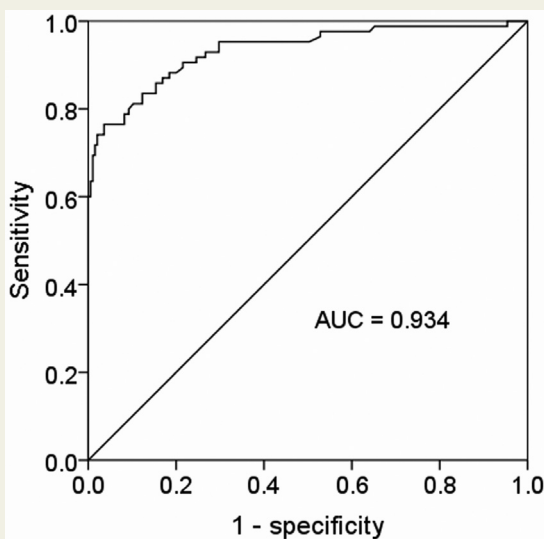


Figure 3 Serum urothelial carcinoma associated 1 can identify patients with acute respiratory distress syndrome from those who underwent cardiopulmonary bypass with the area under the curve (AUC) of 0.934.

with miR-182-5p inhibitor (Figure 5B). However, levels of miR-182-5p did not influence the luciferase activity of cells transfected with mut-UCA1. In addition, the qRT-PCR revealed the downregulation of miR-182-5p in OGD/R-induced cell models, and miR-182-5p levels were elevated after UCA1 downregulation (Figure 5C).

Discussion

With the rapid development of surgical technology, CPB has become increasingly mature. At present, CPB is widely used in clinical practice [24]. However, according to investigation and statistics, the fatality rate of CPB is still as high as 1.7%–3%, and most patients died from respiratory failure, especially ARDS [25,26]. Acute respiratory distress syndrome caused by CPB is a special form of ARDS. Domestic studies have reported that the incidence of ARDS after CPB is as high as 2.5%, leading to a related mortality rate of about 68% [3]. The duration of bypass is known to increase postoperative complications, including ARDS. The current baseline clinical information demonstrated that patients with ARDS had a longer duration of bypass than those without ARDS, revealing its potential association with

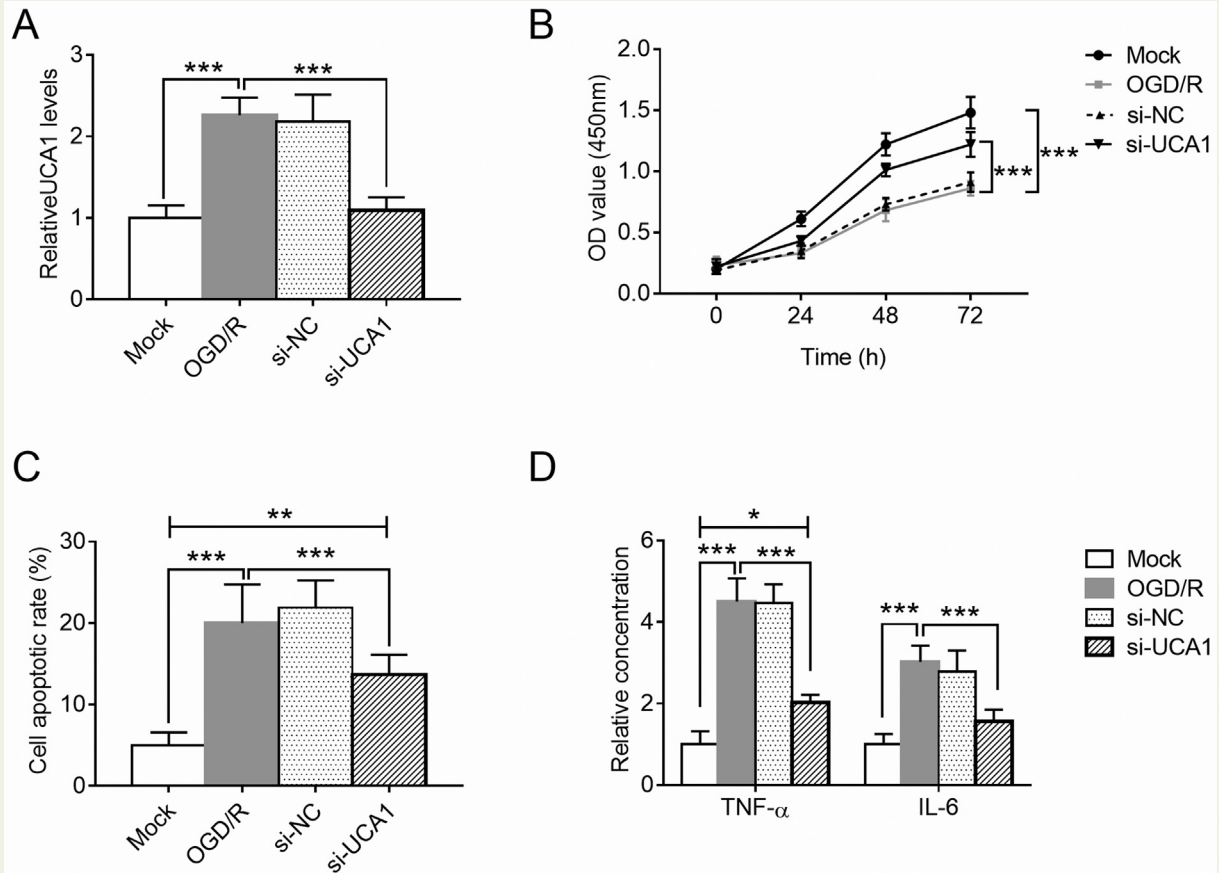


Figure 4 Urothelial carcinoma associated 1 (UCA1) inhibition protects against lung injury and inhibits cell inflammation in vitro. (A) Transfection with small-interfering UCA1 RNA (si-UCA1) inhibits the increased trend of UCA1. (B) si-UCA1 rejuvenated the cell viability inhibition induced by OGD/R. (C) OGD/R-induced cell apoptosis was also recovered by UCA1 block down. (D) si-UCA1 inhibits tumour necrosis factor (TNF)- α and interleukin (IL)-6 release. * $p < 0.05$, *** $p < 0.001$. Abbreviation: si-NC, negative control.

the occurrence of ARDS. Existing studies indicate that using biomarkers can improve the early diagnosis and treatment of ARDS [27]. It can delay or even prevent the progression of high-risk patients to ARDS, which is the key to reducing the incidence and mortality of ARDS, and improving its diagnosis and treatment level [28]. Biomarkers can stratify disease risk under specific conditions, which are of great significance in disease prediction and treatment. As the most widely studied, lncRNA has been proven in recent years to be associated with the pathogenesis of many diseases [29]. Existing studies have shown that lncRNA plays a broad regulatory role in epigenetic and other aspects, and increasing evidence has shown that lncRNA can be used as a biological marker for the diagnosis and treatment of diseases [30]. In the present study, serum UCA1 levels gradually increased in patients after receiving CPB, and patients with ARDS had high levels of UCA1 in comparison to non-ARDS patients at the same time point. In addition, the ROC curve demonstrated the diagnostic ability of serum UCA1 for ARDS. However the retrospective nature of our study made it difficult to collect information of the

ARDS stage, as well as the association of serum UCA1 with disease stage. This was a limitation of our study, and should be considered in future research. In addition, a significantly long duration of bypass was detected in patients with ARDS, and the causal relationship between bypass time and UCA1 levels needs further verification.

The main causes of CPB-induced ARDS include ischaemia-reperfusion-induced injury and activation of systemic inflammatory response [31]. Previous studies have confirmed that the levels of TNF- α and IL-6 are increased in CPB-induced lung injury or ARDS [32]. The level of inflammation may be an important indicator in evaluating the occurrence and development of ARDS [33]. The current ELISA assay consistently demonstrated an increasing trend for TNF- α and IL-6 release in both control and ARDS groups in a time-dependent manner, and patients with ARDS had increased levels of TNF- α and IL-6 vs the control group. The findings reflect the close association between a severe inflammatory response and the development of ARDS. In addition, many studies have used TNF- α and IL-6 levels as biological indicators for

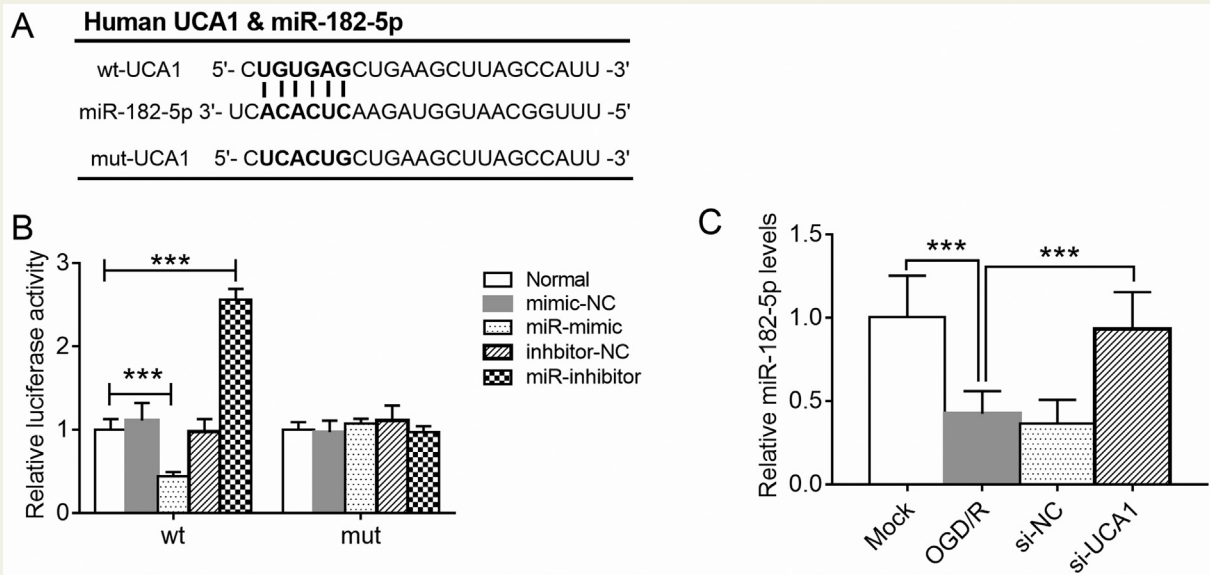


Figure 5 Urothelial carcinoma associated 1 (UCA1) functions through sponging miR-182-5p. (A) Binding sites between UCA1 and miR-182-5p. (B) MiR-182-5p mimics weakened cell luciferase activity. (C) miR-182-5p was downregulated in OGD/R-induced cell models, and elevated after small-interfering UCA1 RNA (si-UCA1) transfection. *** $p < 0.001$. Abbreviations: si-NC, negative control; wt, wild type; mut, mutant.

diagnosis, prediction, and evaluation of lung injury or ARDS [34]. The Pearson correlation analysis in this study demonstrated a close correlation between UCA1 and TNF- α and IL-6 in the serum of patients with ARDS 8 hours after CPB.

It is known that the number and function of alveolar epithelial cells are the functional and structural basis of the lung, and also play a role in the repair of lung injury [35]. In the current study, OGD/R-induced A549 cell models were established for in vitro experiments. Similar to the results observed in patients with ARDS, high levels of UCA1 were also observed in cell models. Besides, cell transfection was performed to regulate UCA1 levels in the cell models. The gain- and loss-of-function experiments demonstrated that UCA1 knockdown protected against OGD/R-induced cell apoptosis and inflammatory response. The findings led us to conclude that UCA1 is an important factor in the development of ARDS.

UCA1 has been reported to regulate the occurrence and development of human diseases by acting as a molecular sponge of miRNA [36]. Previous evidence and the present findings have highlighted the target relationship between UCA1 and miR-182-5p. The present cell experiments showed the downregulation of miR-182-5p in OGD/R-induced cell models. Consistently, miR-182-5p has been widely reported to be involved in lung disease, such as acute lung injury and lung cancer [20,21]. We speculated that miR-182-5p might be related to the regulatory role of UCA1 in the development of ARDS.

In conclusion, patients who received CPB had increased levels of UCA1, and high levels of UCA1 were associated with the occurrence of postoperative ARDS. The regulatory role of UCA1 in ARDS might be related to inflammation and downregulated miR-182-5p in alveolar epithelial cells.

Conflicts of Interest

There are no conflicts of interest to disclose.

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